



A Comparative Cross Study of Robotic Extraction Platforms for Casework and Research Applications – Assessing the AutoMate *Express*[™]

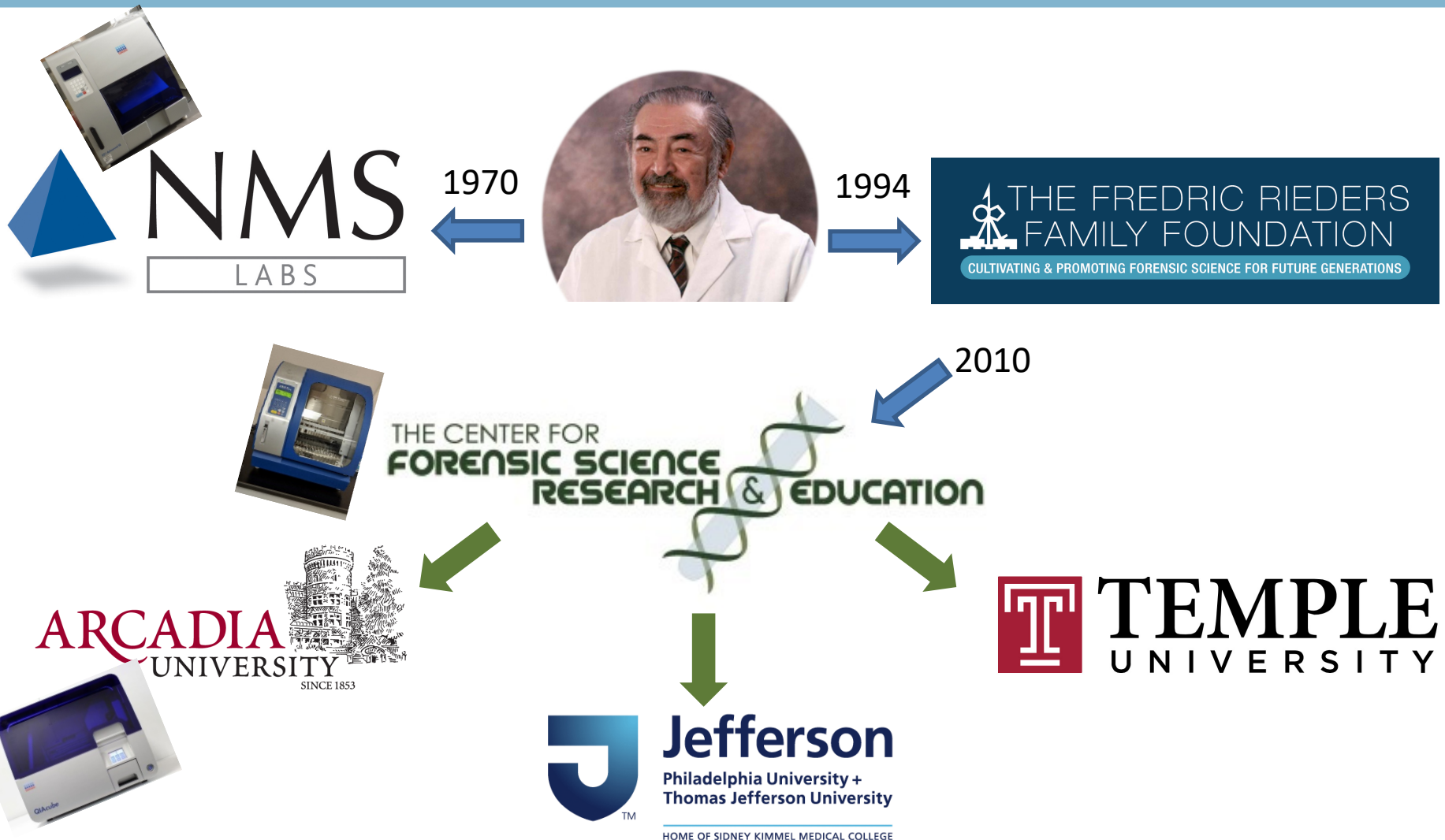
Heather V. Milnthorp

Thermo Fisher 2018 *Future Trends in Forensic DNA Technology*

Foxwoods Resort & Casino – Mashantucket, Connecticut

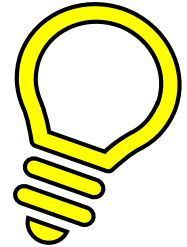
June 1st, 2018

About Our Organization



Why Choose Automation?

- Kits are already optimized for forensic processing!
- Nucleic acids can be purified from a variety of sample types
- Minimizes sample handling and contamination risks
- Less hands on time for the technician/analyst



Applied Biosystems®

AutoMate Express™ DNA Extraction System



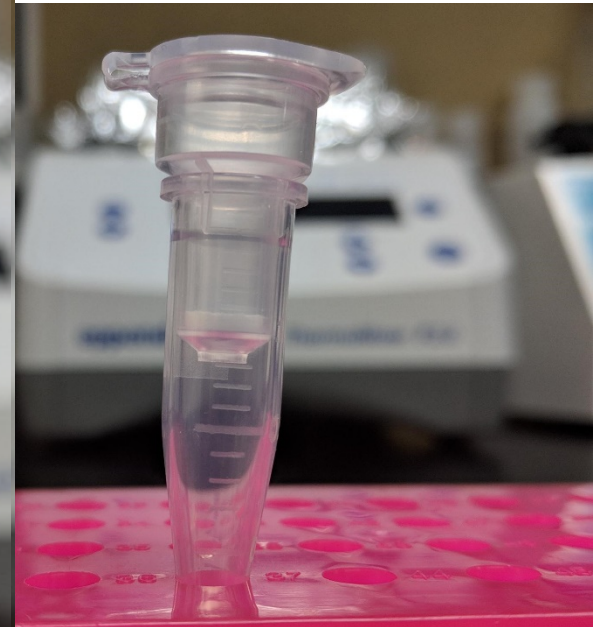
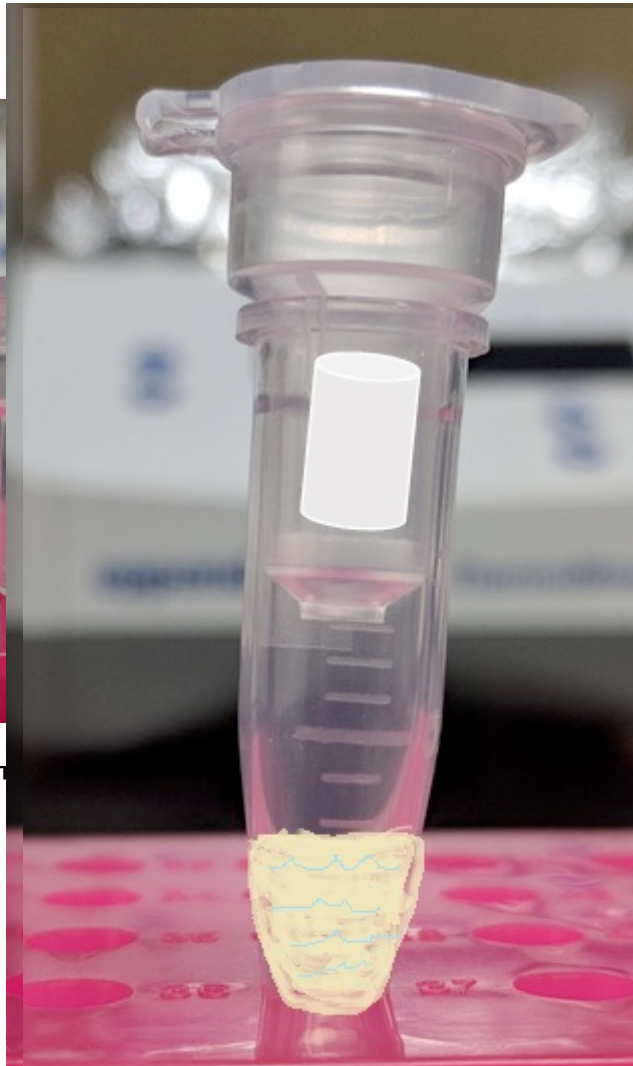
- Compatible kits
 - PrepFiler Express™
 - PrepFiler Express BTA™
- N = 13
- Recommended incubation time = 40 minutes
- Platform run time = < 30 minutes
- Elutes in buffer
 - 7 variable elution volume options

PrepFiler LySep™ Column



1.

ep



Assembled PrepFiler
LySep™ Column

QIAGEN®

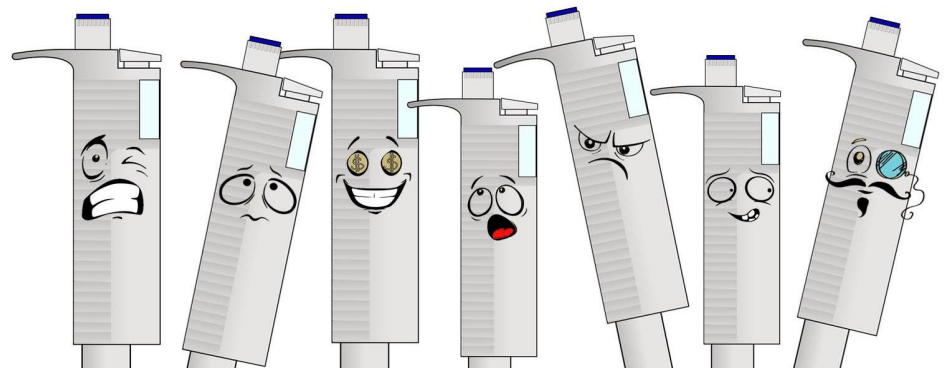
EZ1® Advanced XL



- Compatible kits
 - Many!
 - Forensic: EZ1 DNA Investigator Kit
- N = 14
- Recommended incubation time = sample type dependent
 - NMS validated incubation time = 15 minutes
- Platform run time = < 20 minutes
- Elutes in buffer or water
 - 4 variable elution volume options

Additional Features

- UV lamp
- Direct supply of carrier RNA
 - Also supplies G2 Buffer and Proteinase K
- Additional protocol options
 - Trace Protocol
 - Large Volume Protocol
 - “Tip Dance” protocol

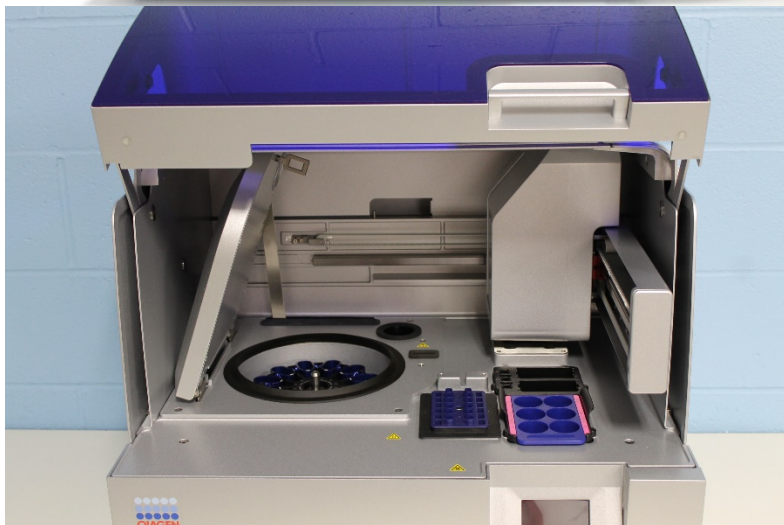


QIAGEN®

QIAcube® System

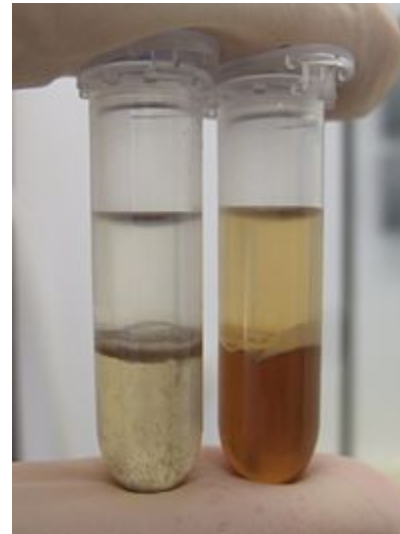


- Compatible kits
 - Most manual kits
 - Forensic: QIAamp DNA Investigator Kit
- N = 12
- Recommended incubation time depends on protocol / sample type
 - 1 hour on robot
- Platform run time = > 75 minutes*
- Elutes in buffer or water
 - 20-100µL



Additional Non-Robotic Extractions Assessed

- PrepFiler™ Forensic DNA Extraction Kit
- Organic Extraction



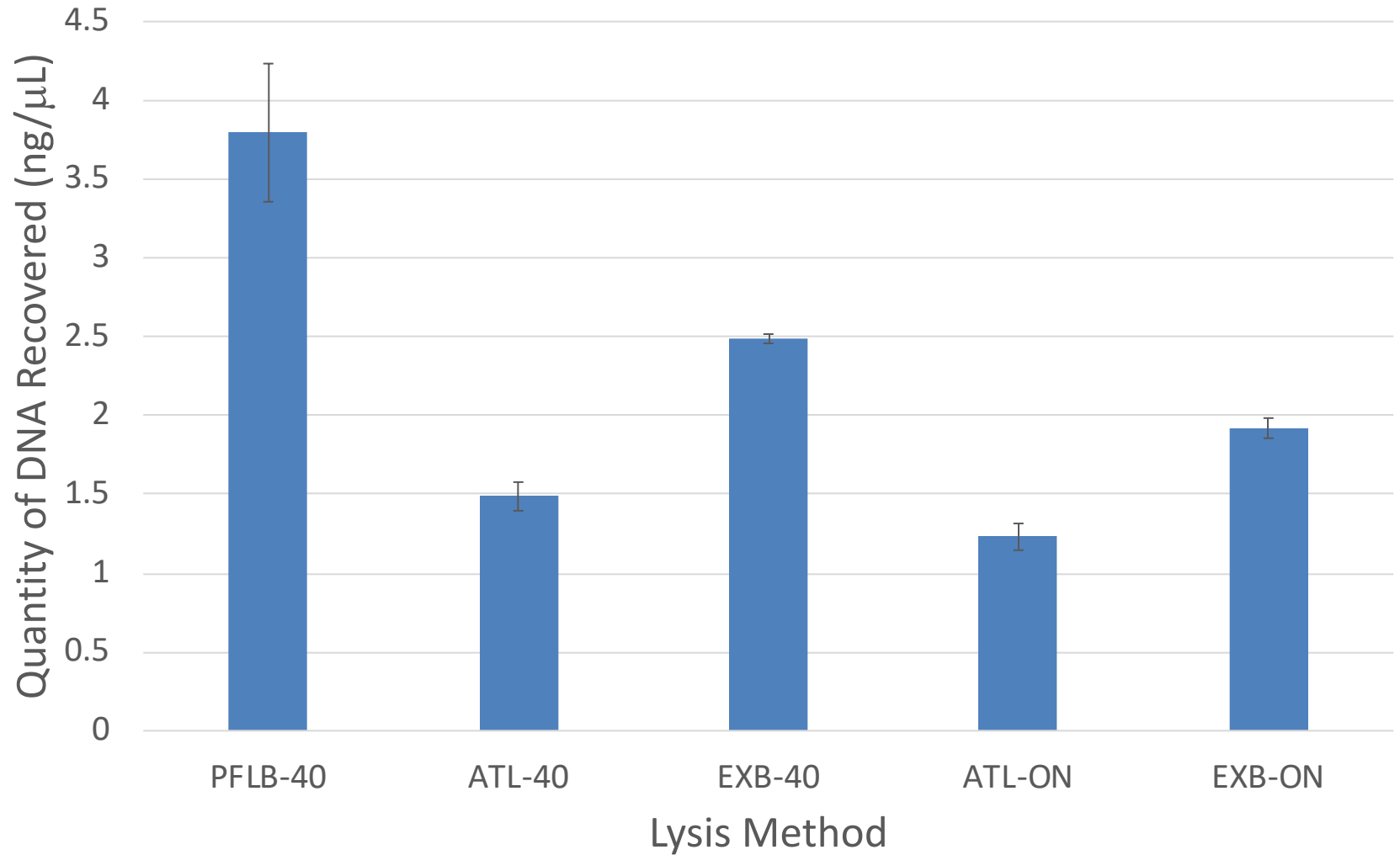
Outline of “AutoMate” Project

- Brief lysis optimization study for the AutoMate
 - Length of digest, composition of digest buffer
- Comparative study to other robotic extraction platforms and manual extraction techniques
 - Sensitivity Study
 - Case-Type Samples
- Assessment of variable elution volumes for research applications
- Development of differential extraction protocol

Lysis Optimization for the AutoMate™ *Express*

- Compare different buffer formulations
 - PrepFiler™ Lysis Buffer + 1M DTT
 - QIAGEN® Buffer ATL + Proteinase K
 - Homebrew Extraction Buffer
 - TE-4 Buffer, ATL Buffer, 1M DTT, and Proteinase K
- Compare alternate incubation lengths
 - 40 Minutes
 - Overnight (16 Hours)

PrepFiler Express™ Lysis Optimization



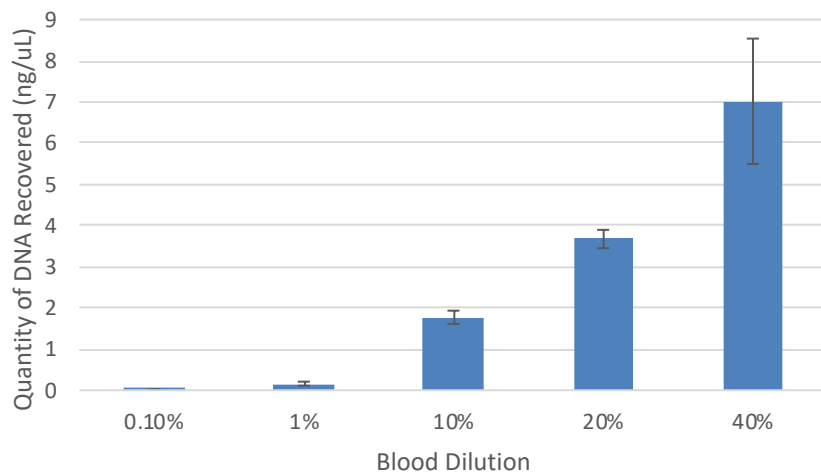
Sensitivity

- 20 microliter aliquots of serial blood dilutions
 - 0.1%
 - 1%
 - 10%
 - 20%
 - 40%
- 5 replicates of each dilution were extracted per method

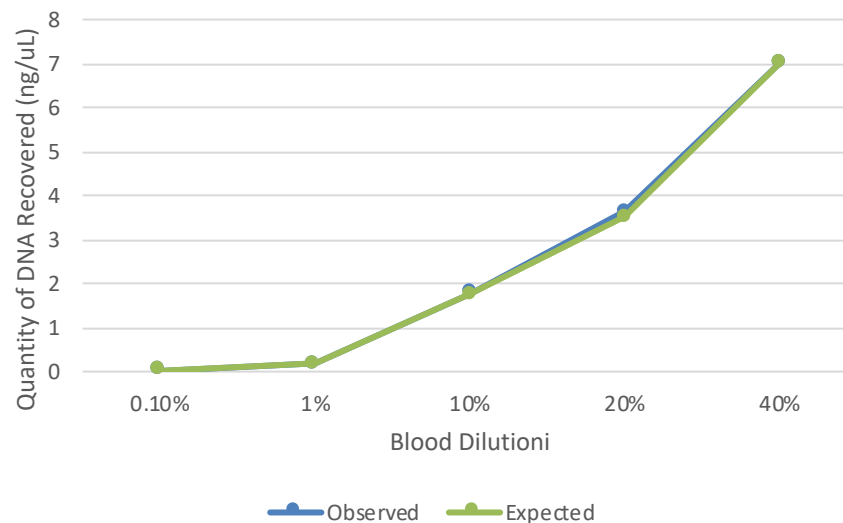


PrepFiler Express™ Kit

Automate Express - PrepFiler Kit



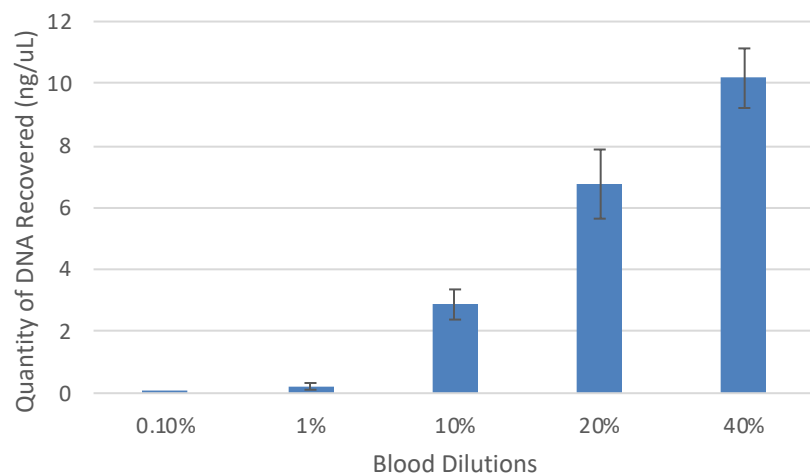
Linearity - PrepFiler Kit



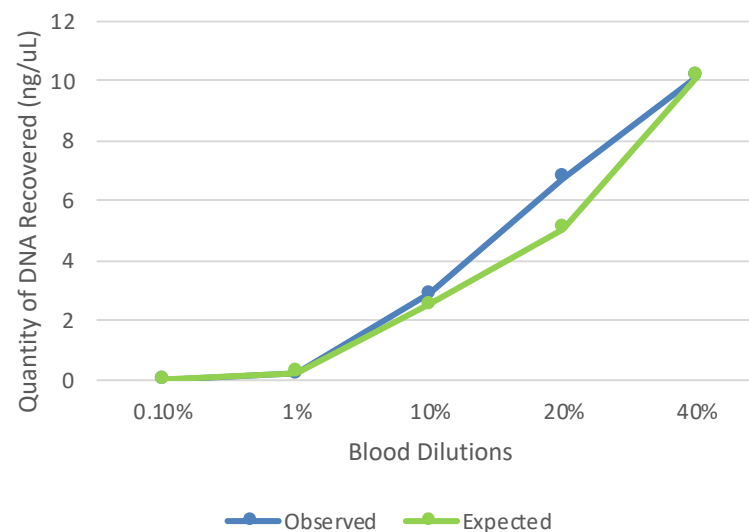
	0.1%	1%	10%	20%	40%
Mean	0.013309	0.159344	1.772271	3.670972	7.016315
Std. Dev	0.003543	0.049541	0.16018	0.22359	1.521231

PrepFiler™ Kit (Manual)

PrepFiler Manual Extraction



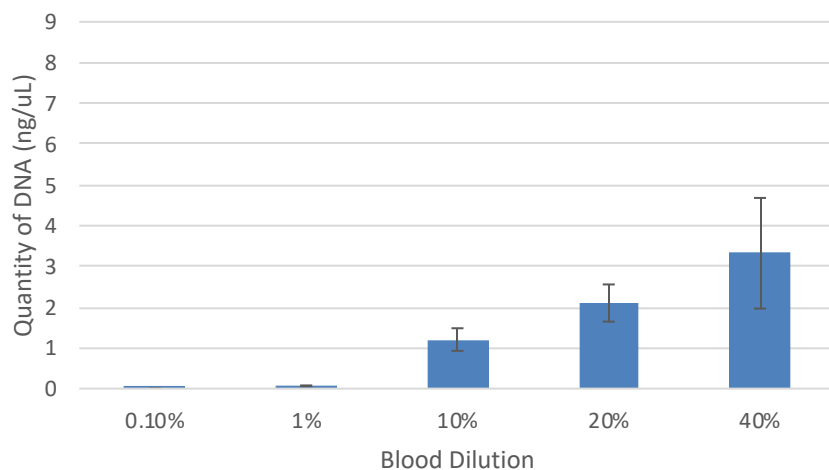
Linearity - PrepFiler Manual Extraction



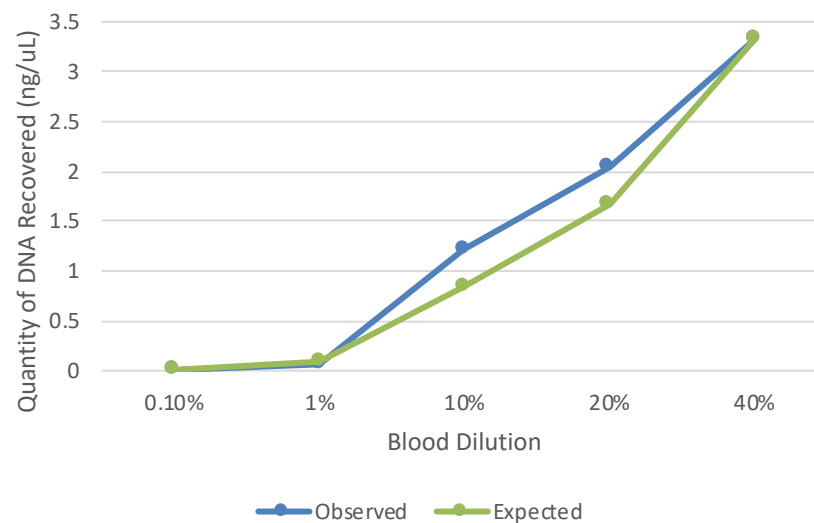
	0.1%	1%	10%	20%	40%
Mean	0.011193	0.220568	2.863981	6.751921	10.17035
Std. Dev	0.001196	0.10863	0.486991	1.116575	0.96186

PrepFiler Express BTA™ Kit

Automate Express - BTA Kit



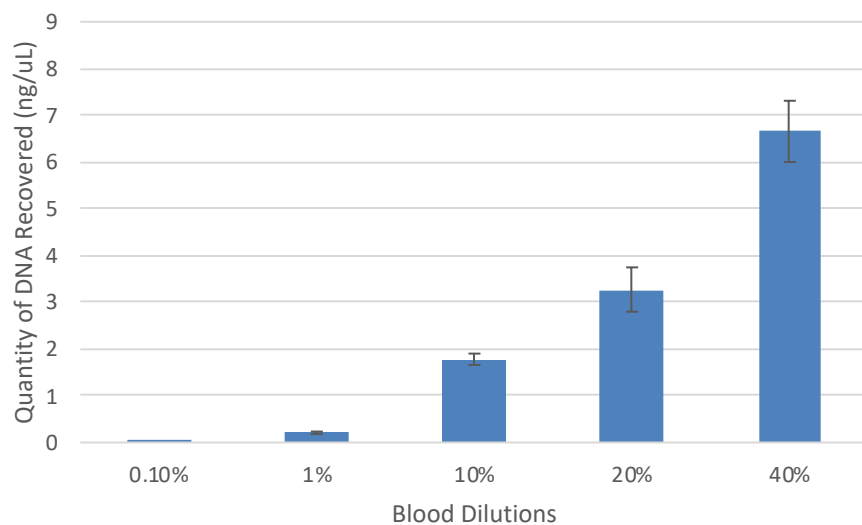
Linearity - BTA Kit



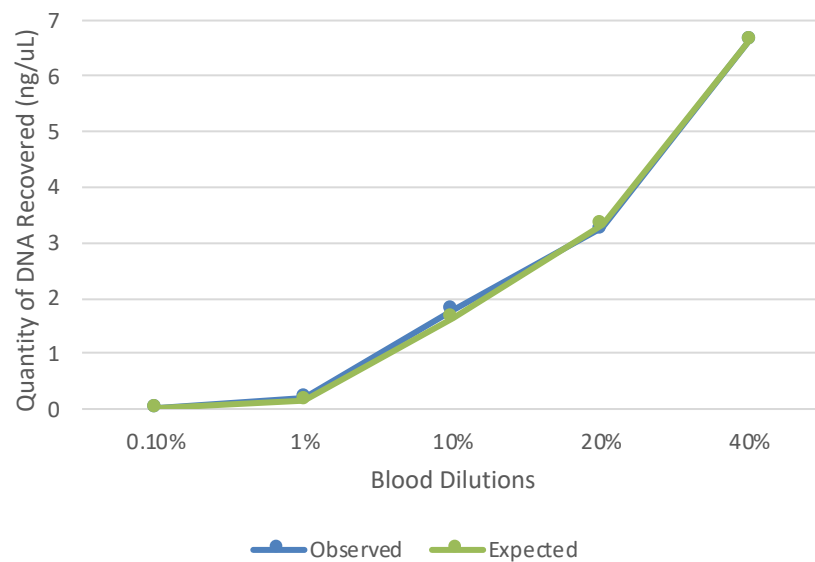
	0.1%	1%	10%	20%	40%
Mean	0.005653	0.066038	1.203766	2.100319	3.321876
Std. Dev	0.002619	0.011934	0.277554	0.456226	1.357928

EZ1[®] DNA Investigator Kit

EZ1 Investigator Kit



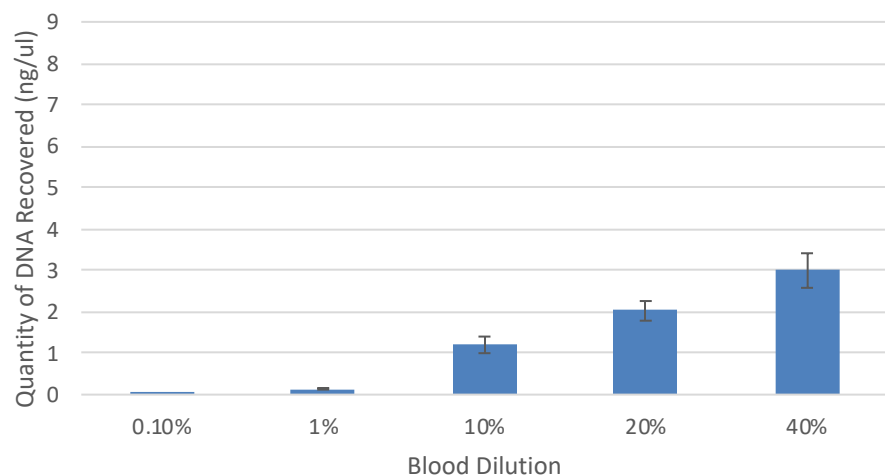
Linearity - EZ1 Investigator Kit



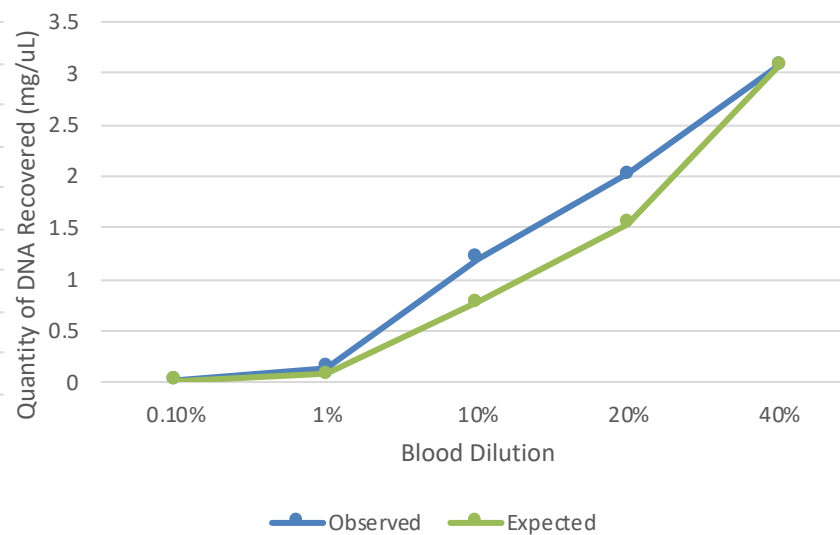
	0.1%	1%	10%	20%	40%
Mean	0.018249	0.207581	1.780472	3.272231	6.655823
Std. Dev	0.007695	0.027235	0.121364	0.474615	0.654902

QIAamp[®] DNA Investigator Kit

QIACube - DNA Investigator Kit



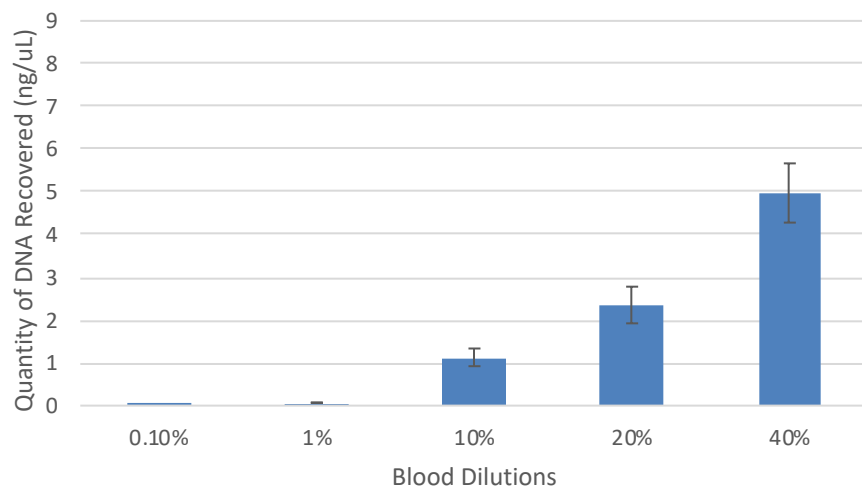
Linearity - QIACube



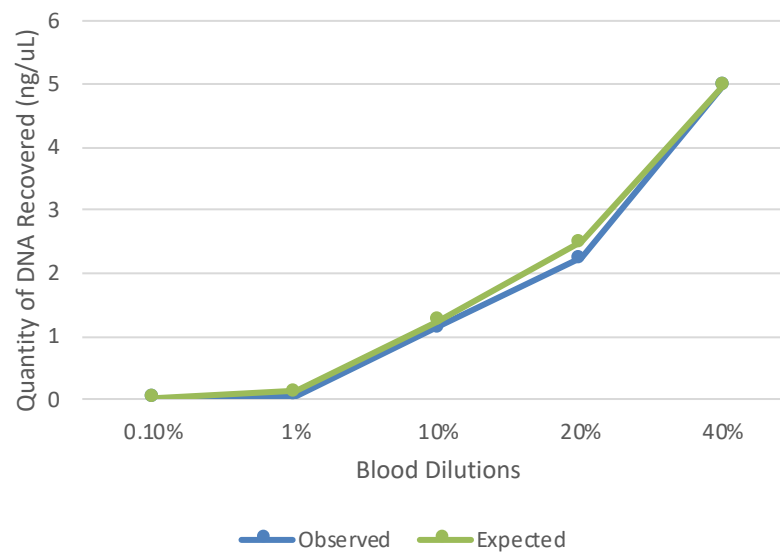
	0.1%	1%	10%	20%	40%
Mean	0.016872	0.140092	1.200939	2.022246	3.08273
Std. Dev	0.003748	0.019389	0.200507	0.237252	0.333108

Manual Organic Extraction

Organic Extraction (PCIA)

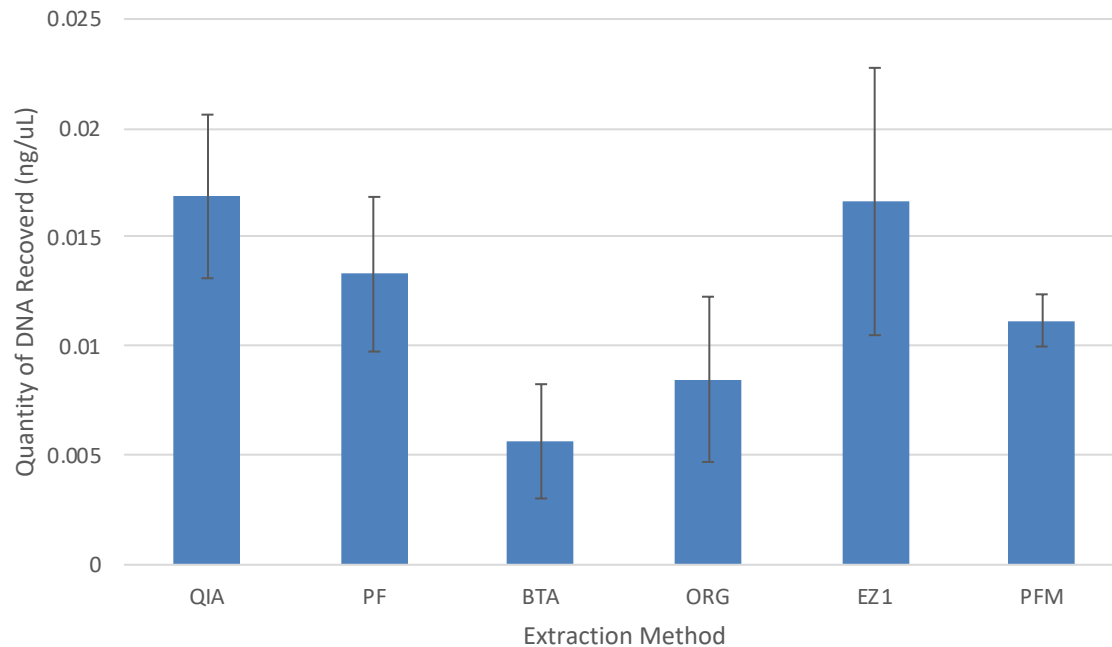


Linearity - Organic Extraction



	0.1%	1%	10%	20%	40%
Mean	0.008494	0.068299	1.128937	2.353379	4.973985
Std. Dev	0.003786	0.007713	0.20709	0.42894	0.69275

Performance with a 0.1% Blood Dilution



Single Factor ANOVA

$F_S = 12.8183$

$F_C = 2.57874$

$P = 4.8E-07$

SIGNIFICANT DIFFERENCE

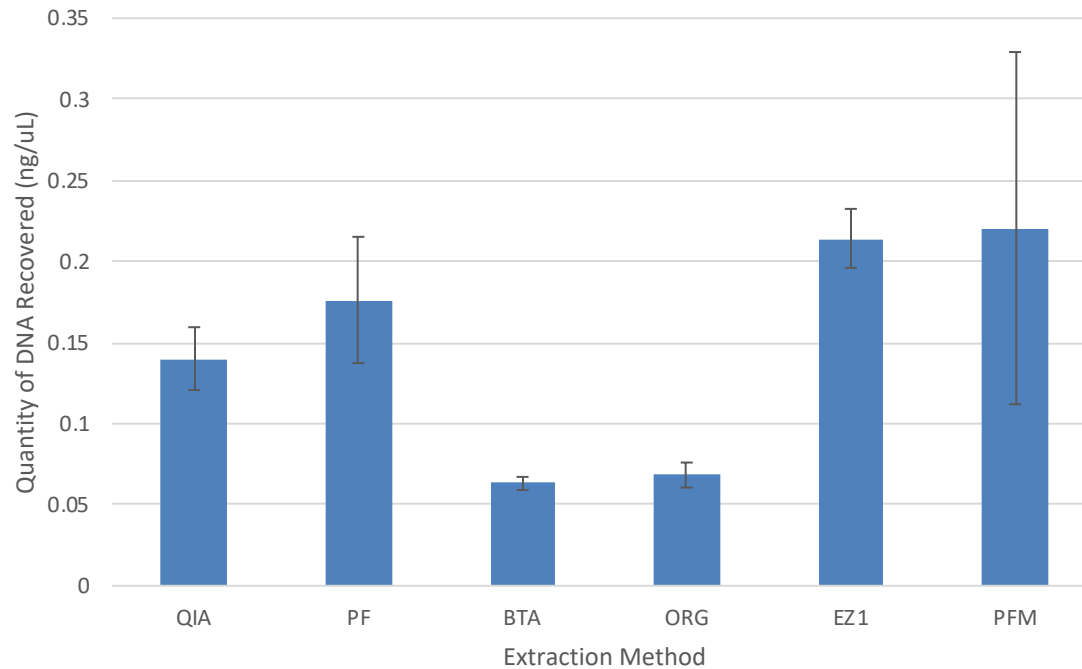
Post-Hoc Tukey Test

QIA >> BTA, ORG ($p=0.01$)

PF > BTA ($p=0.05$)

EZ1 >> BTA, ORG ($p=0.01$)

Performance with a 1% Blood Dilution



Single Factor ANOVA

$$F_S = 49.2357$$

$$F_C = 2.57874$$

$$P = 7.1E-16$$

SIGNIFICANT DIFFERENCE

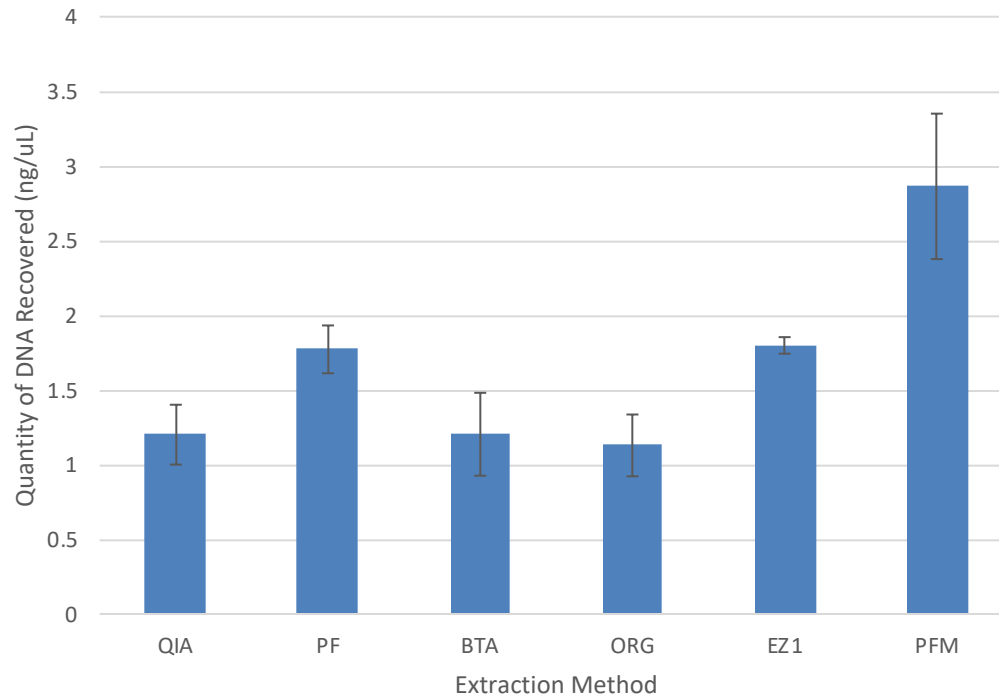
Post-Hoc Tukey Test

QIA >> BTA, ORG (p=0.01)

PF >> BTA, ORG (p=0.01)

EZ1 >> QIA, BTA, ORG (p=0.01)

Performance with a 10% Blood Dilution



Single Factor ANOVA

$$F_S = 26.0263$$

$$F_C = 2.58884$$

$$P = 5.3E-11$$

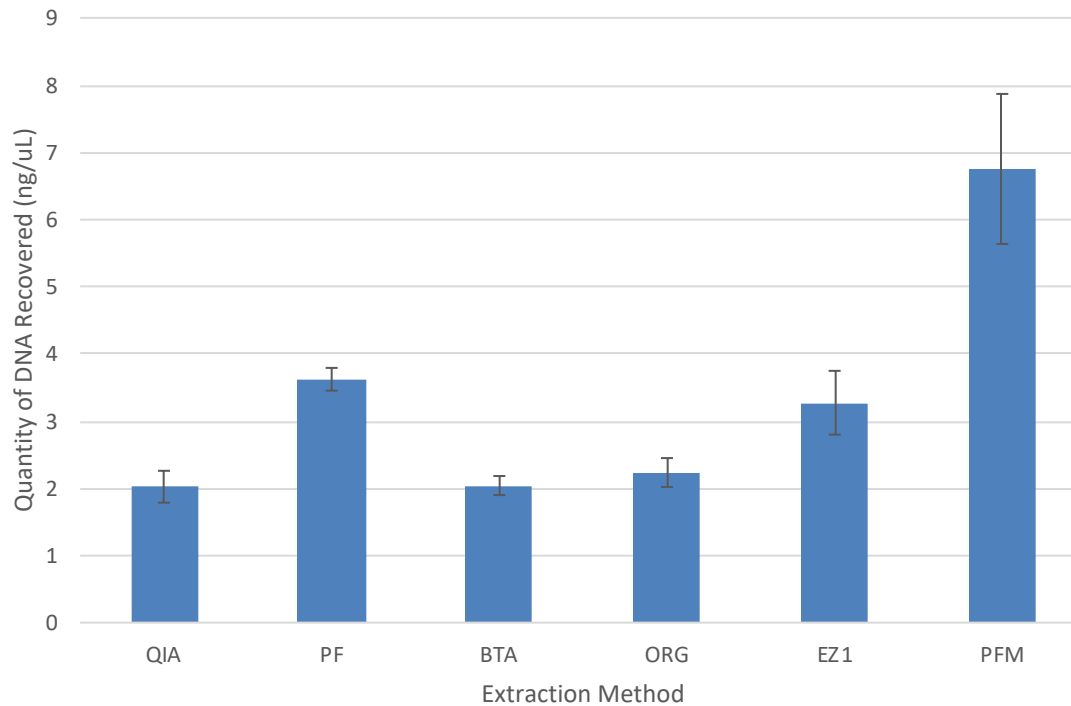
SIGNIFICANT DIFFERENCE

Post-Hoc Tukey Test

PF >> QIA, BTA, ORG (p=0.01)

EZ1 >> QIA, BTA, ORG (p=0.01)

Performance with a 20% Blood Dilution



Single Factor ANOVA

$$F_S = 37.8738$$

$$F_C = 2.58884$$

$$P = 1.4E-13$$

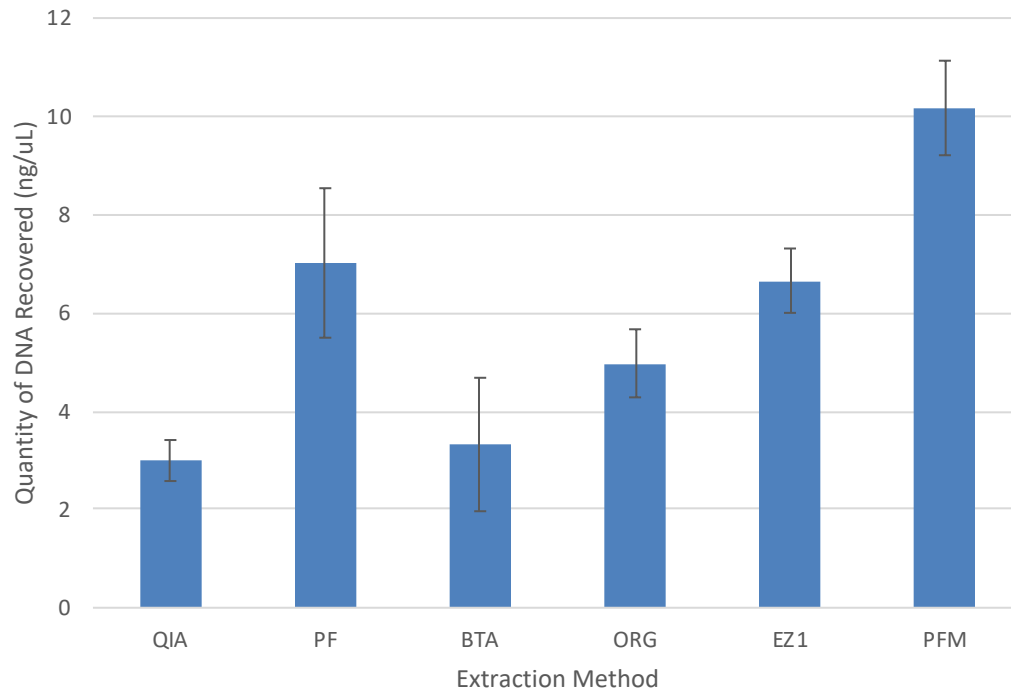
SIGNIFICANT DIFFERENCE

Post-Hoc Tukey Test

PF >> QIA, BTA, ORG ($p=0.01$)

EZ1 >> QIA, BTA, ORG ($p=0.01$)

Performance with a 40% Blood Dilution



Single Factor ANOVA

$$F_S = 32.2855$$

$$F_C = 2.58884$$

$$P = 1.9E-12$$

SIGNIFICANT DIFFERENCE

Post-Hoc Tukey Test

PF >> QIA, BTA, ORG (p=0.01)

ORG >> QIA, BTA (p=0.01)

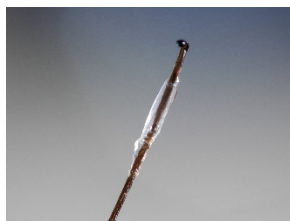
EZ1 >> QIA, BTA, ORG (p=0.01)

Sensitivity Study: Take Home Message

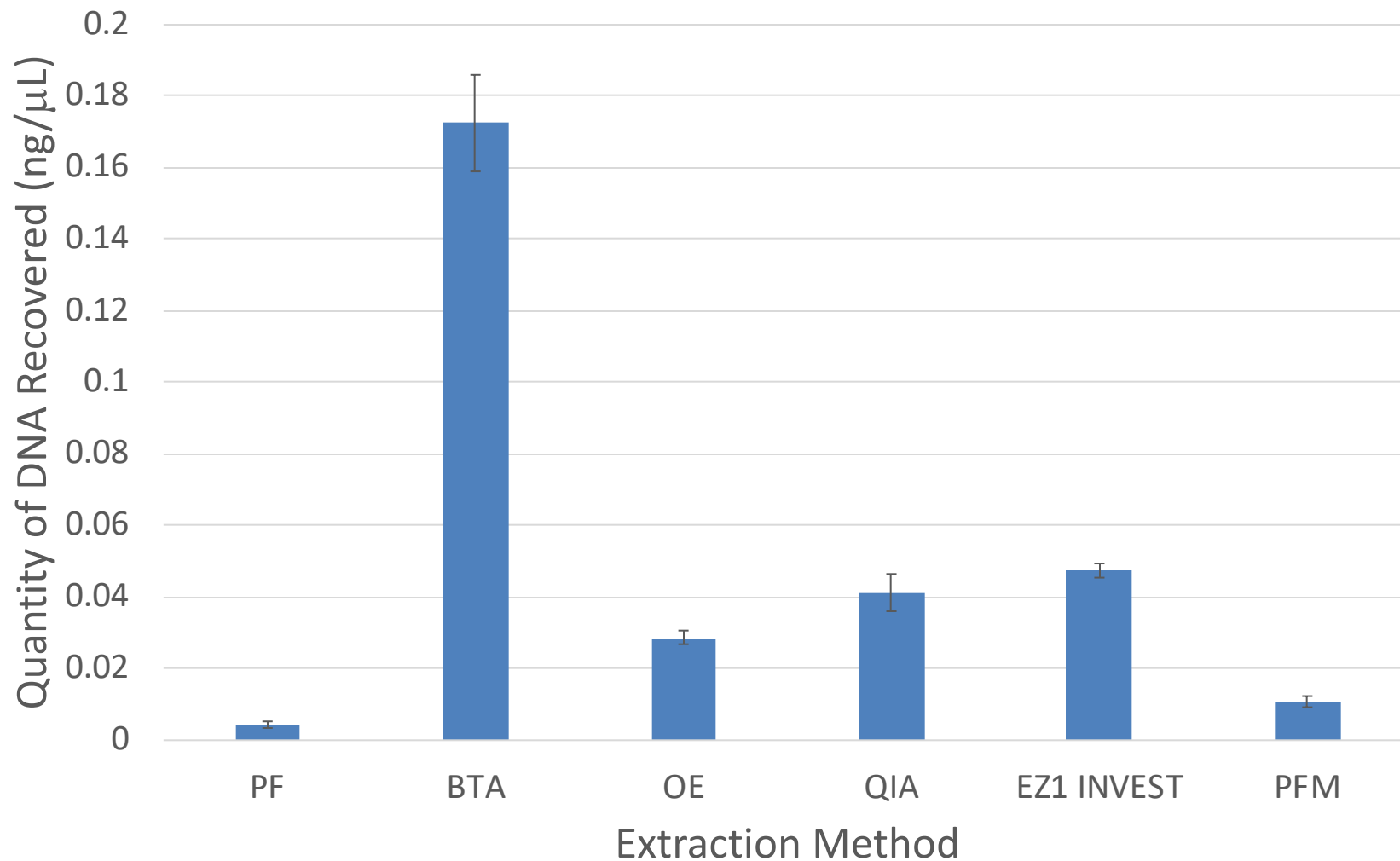
- For blood samples...
 - PrepFiler *Express*[™] >>> PrepFiler *Express* BTA[™]
 - PrepFiler *Express*[™] and EZ1[®] DNA Investigator Kit
>>> Organic Extraction and QIAamp DNA Investigator Kit

Case-Type Samples

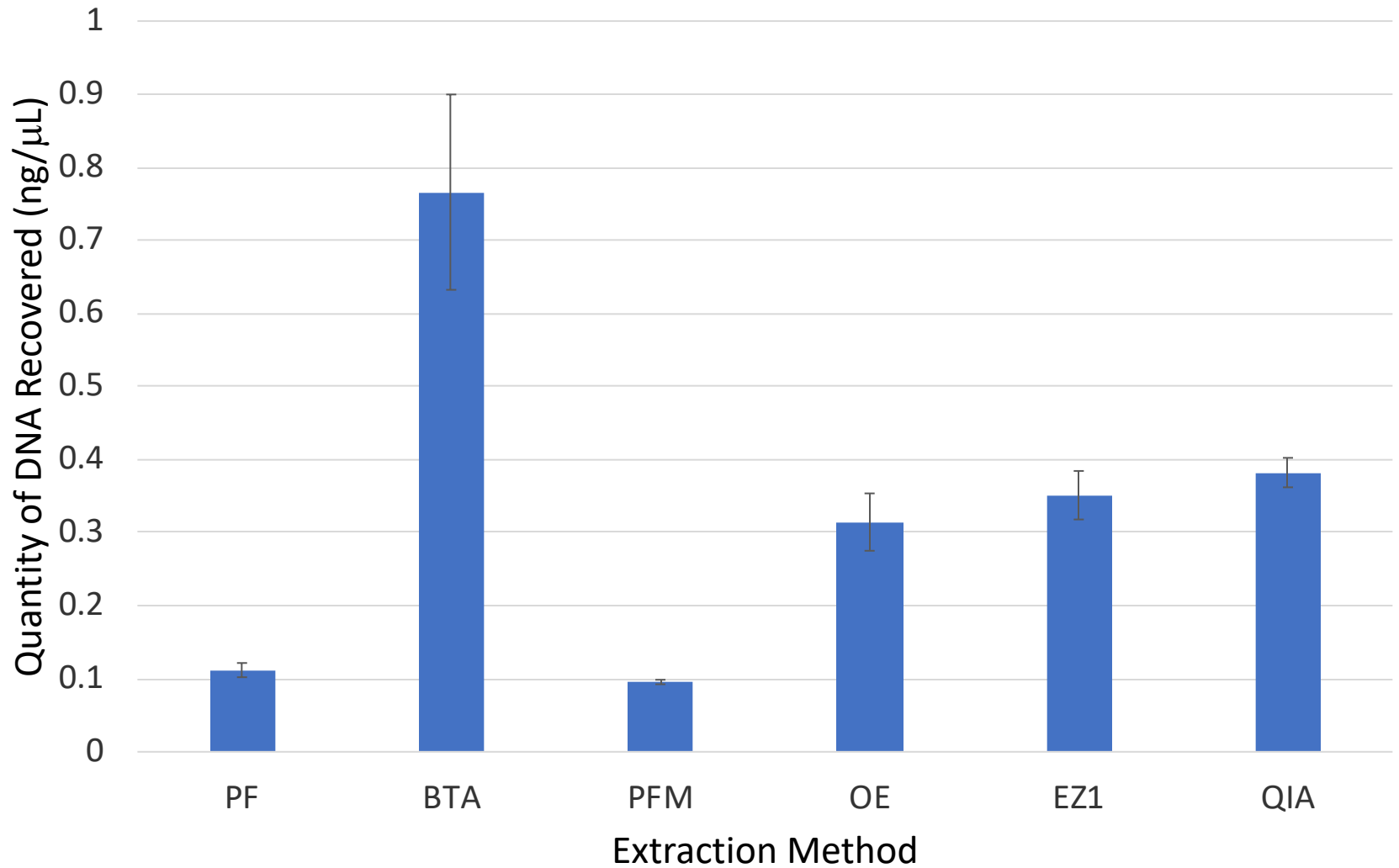
- Touch samples
 - 10 per method; all external firearms swabbings
- Low level saliva on cotton swatch
- Semen/Vaginal fluid mixture
- Low level semen on fabric
- Low level semen + lubricant on cotton swab
- Blood on denim
- Blood + humic acid solution
- Blood + bleach
- Hair with root
- Cigarettes
 - Unsmoked with saliva placed on to the filter
 - Smoked



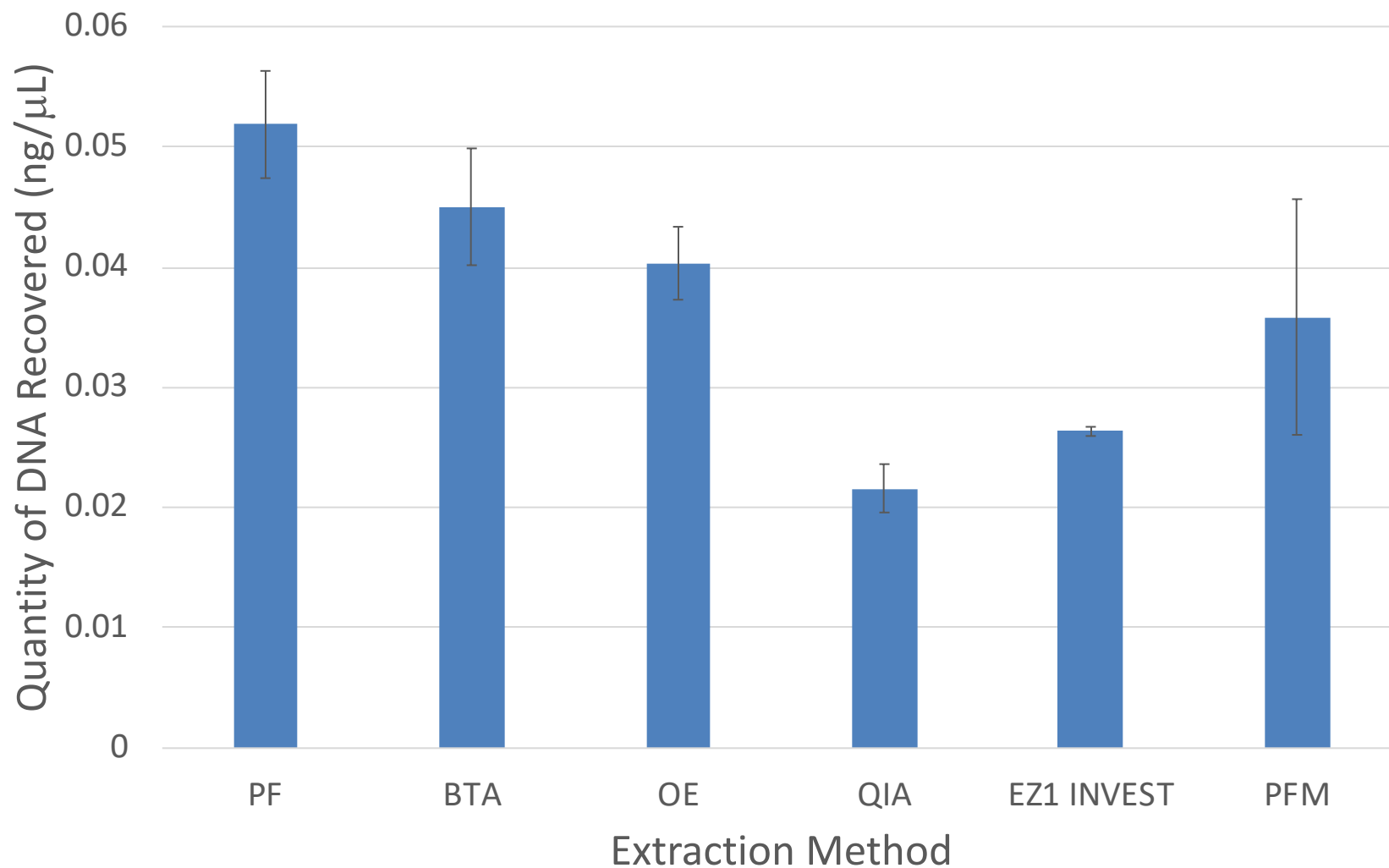
Unsmoked Cigarette Butt + 20 μ L saliva



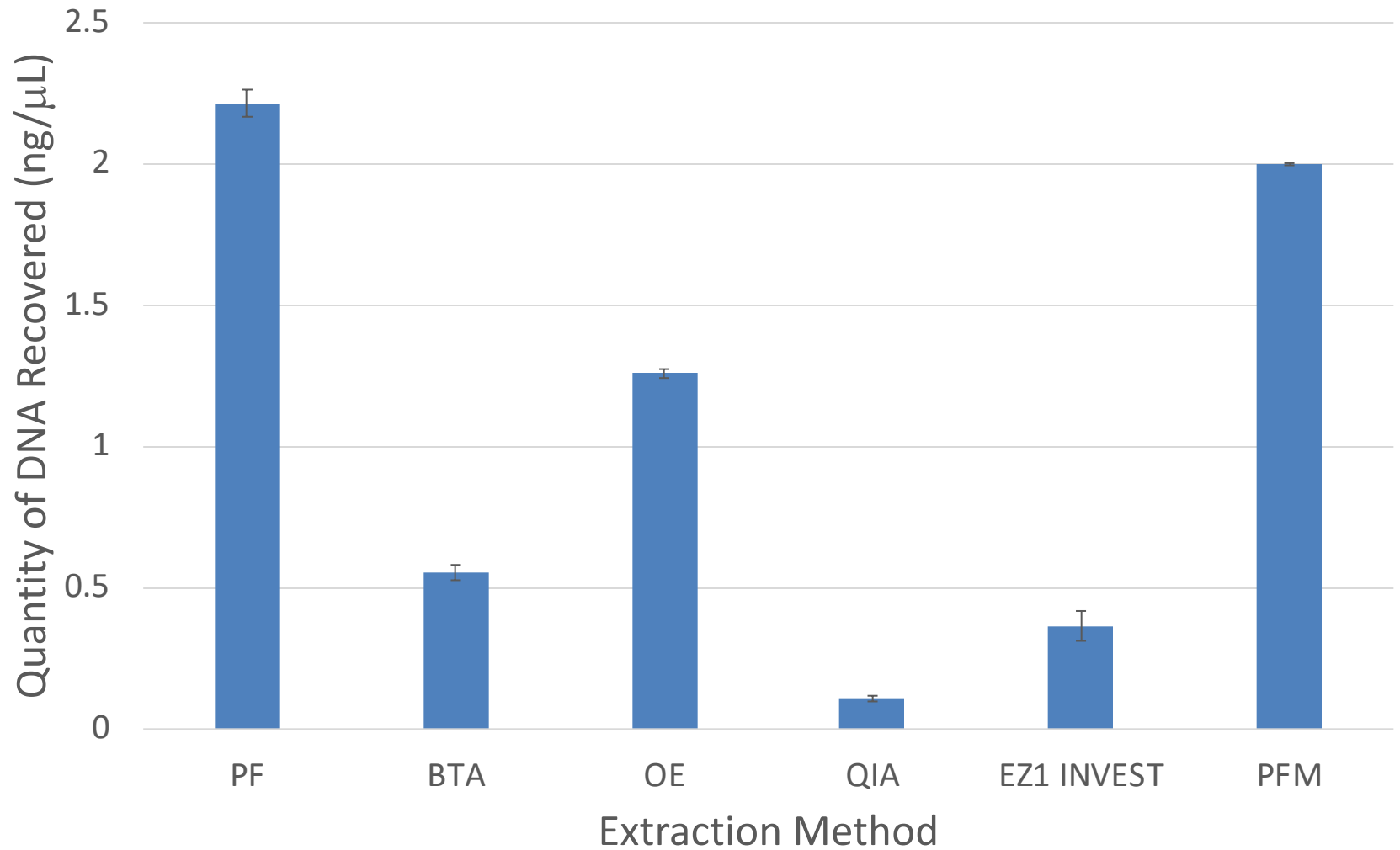
Cutting From a Smoked Cigarette Butt



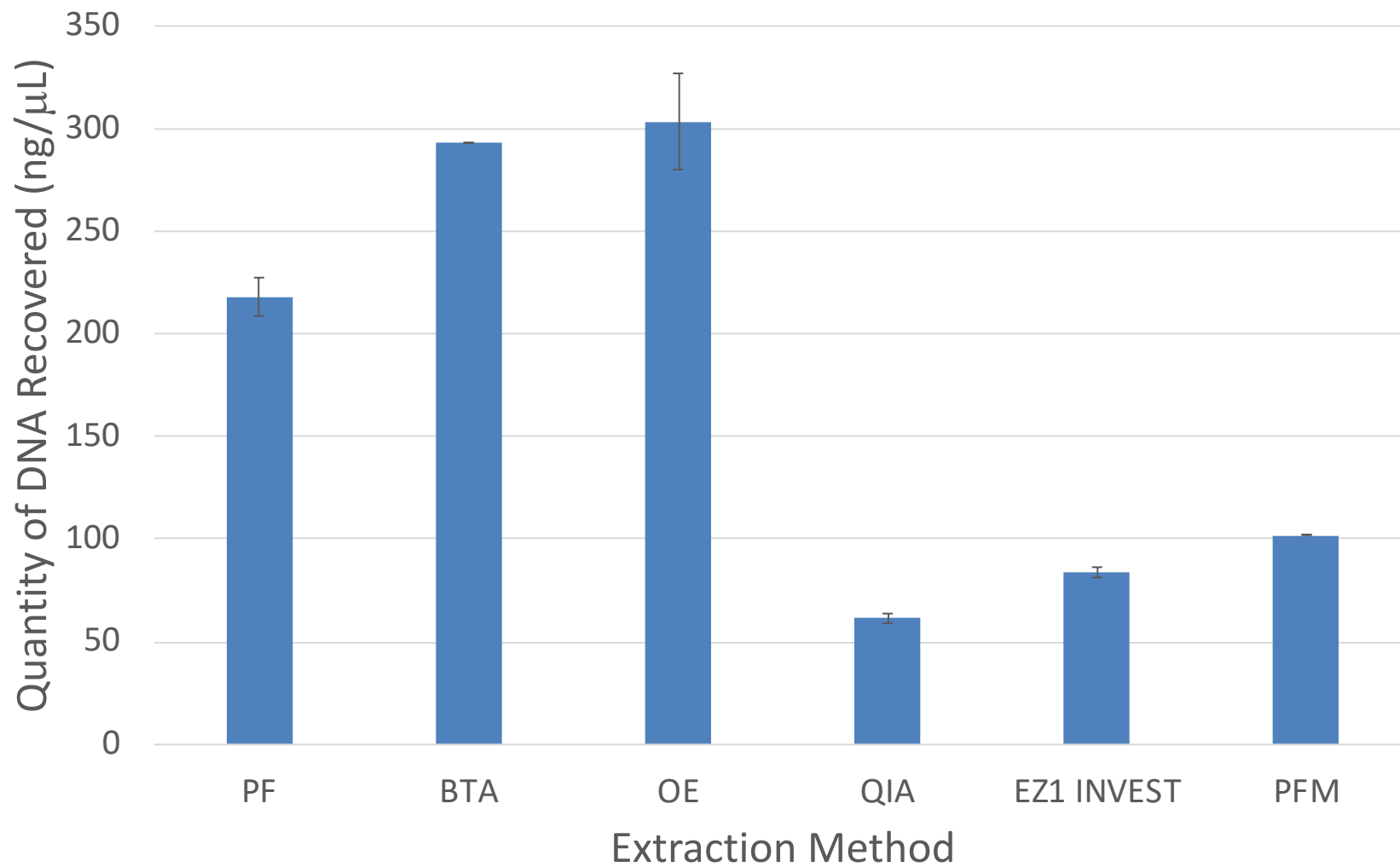
Low Level Semen on Cotton Swab (100 μ L of 1:2000 dilution)



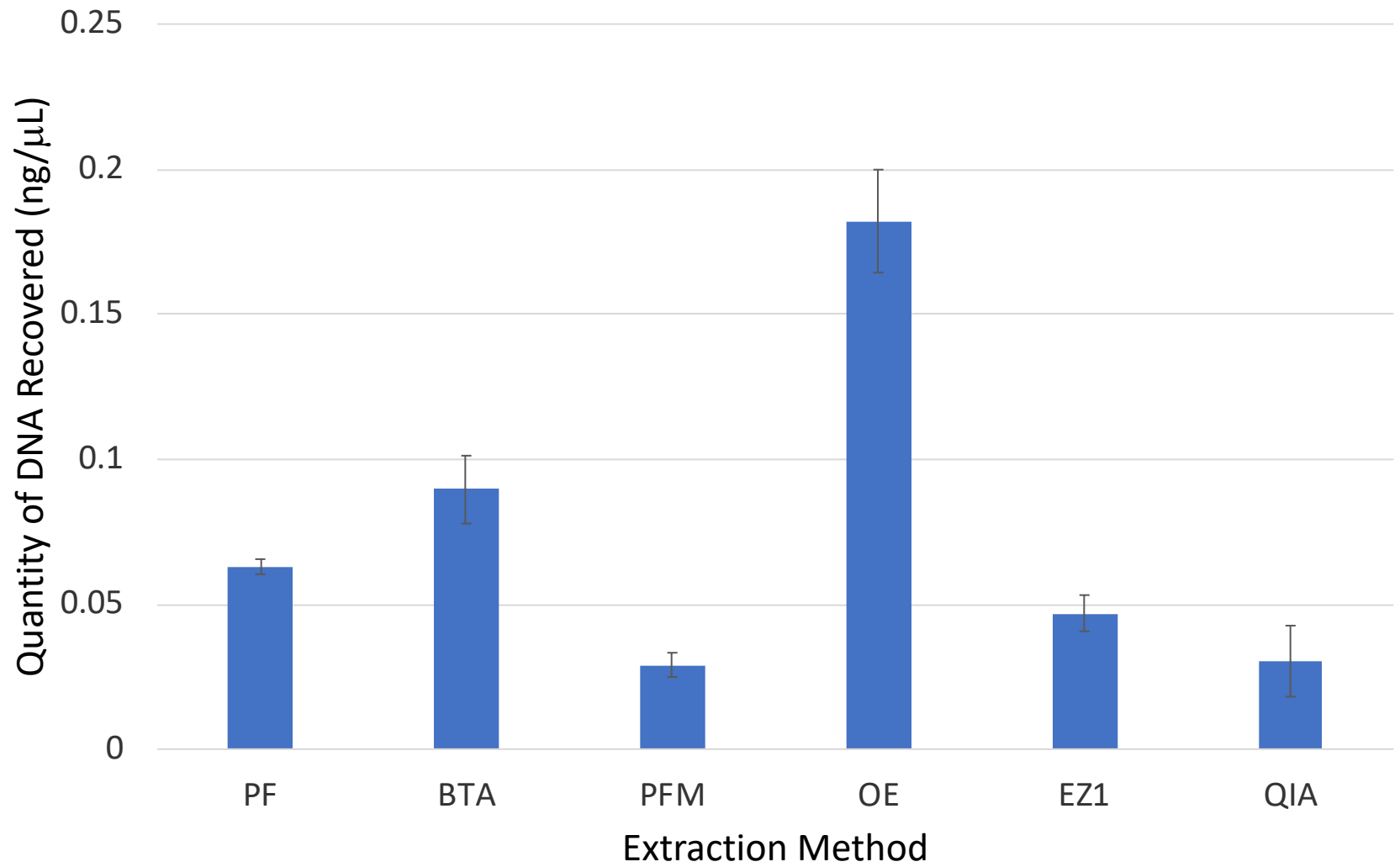
5 μ L neat blood + 1 μ L 2ng/mL humic acid solution



5 mL of neat semen on a vaginal swab



Low Level Semen with Lubricant



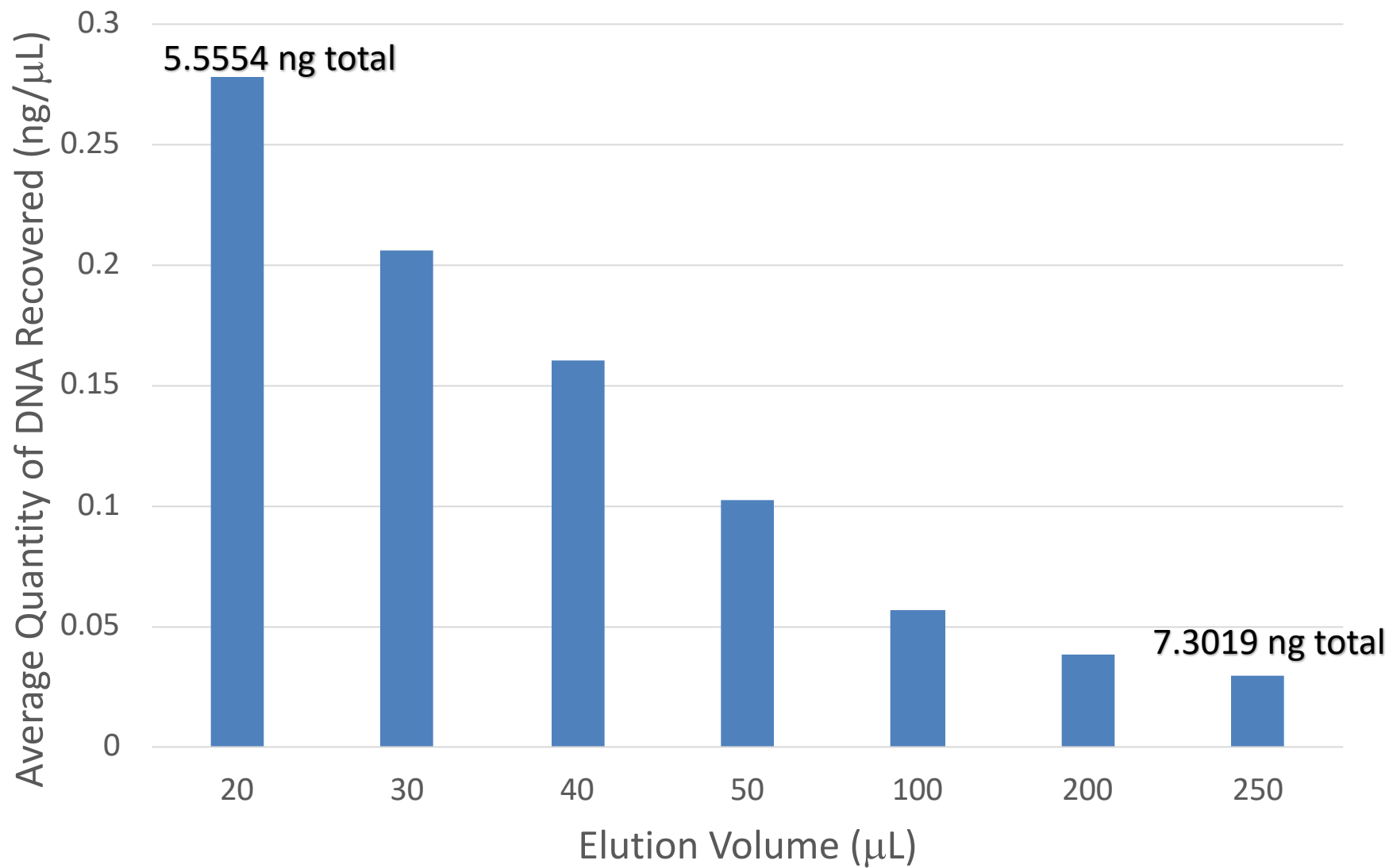
Platform Highlights

	AutoMate™ Express	EZ1® Advanced XL
Samples	13	14
Maximum Lysate Volume	500	200/500
Incubation Time	40 minutes	15+ minutes
Run Time	30 minutes	20 minutes
Elution Media	Buffer	Buffer / H2O
Elution Options	7	4
Instrument List Price	~\$44,000	~\$46,000
Kit Size	52	48
Kit List Price	\$504 / \$548	\$498

Variable Elution Volumes

- Interested in assessing the variable elution volumes for research applications
 - In-House optimized protocols for challenging sample types utilizes a consumptive dry-down of the extract
 - Will we get more total DNA with a higher elution volume?

Assessment of Variable Elution Volumes



Differential Extraction Protocol

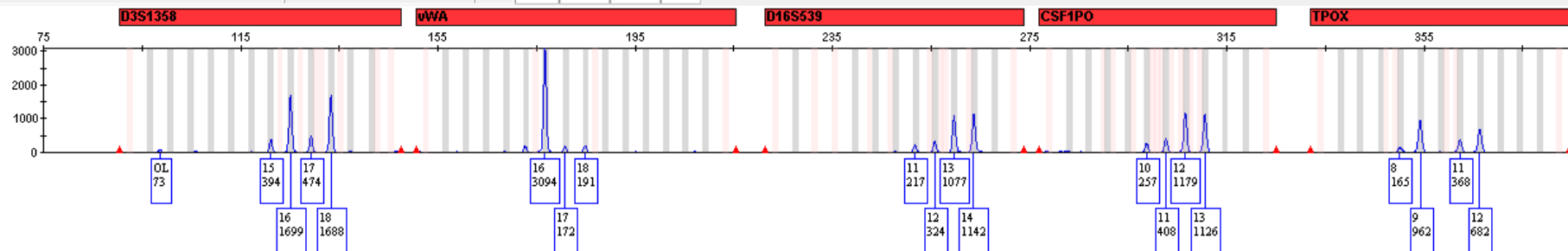
- Examine effects of...
 - Increasing number of sperm pellet wash steps
 - Single lysis vs. double lysis
 - Feasibility of using LySep™ Columns for initial digest

Comparison of Lysis Workflows

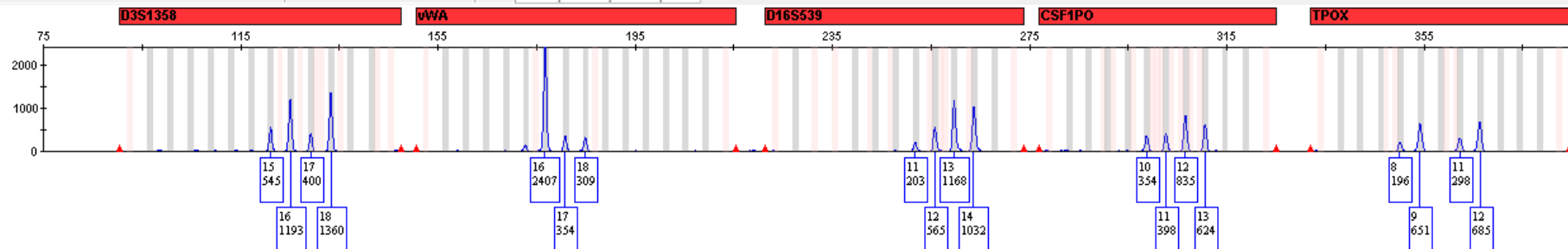
DE1	Lysis 1 - 40 min	full speed 5 min	remove EF	resuspend w/ 50 UPH2O and transfer	1 wash	Lysis 3	
DE2	Lysis 1 - 40 min	full speed 5 min	remove EF	resuspend w/ 50 UPH2O and transfer	2 wash	Lysis 3	
DE3	Lysis 1 - 40 min	full speed 5 min	remove EF	resuspend w/ 50 UPH2O and transfer	3 wash	Lysis 3	
DE4	Lysis 1 - 30 min	full speed 5 min	remove EF	resuspend w/ 50 UPH2O and transfer	Lysis 2	1 wash	Lysis 3
DE5	Lysis 1 - 30 min	full speed 5 min	remove EF	resuspend w/ 50 UPH2O and transfer	Lysis 2	2 wash	Lysis 3
DE6	Lysis 1 - 30 min	full speed 5 min	remove EF	resuspend w/ 50 UPH2O and transfer	Lysis 2	3 wash	Lysis 3

<u>Lysis 1</u>	<u>Lysis 2</u>	<u>Lysis 3</u>
490 EXB	390 EXB	470 PFLB
10 ProK	10 proteinase K	20 DTT
56 deg C	56 deg C	10 proteinase K
900 RPM	900 RPM	70 deg C
40 or 30 minutes	15 minutes	750 RPM
		40 minutes

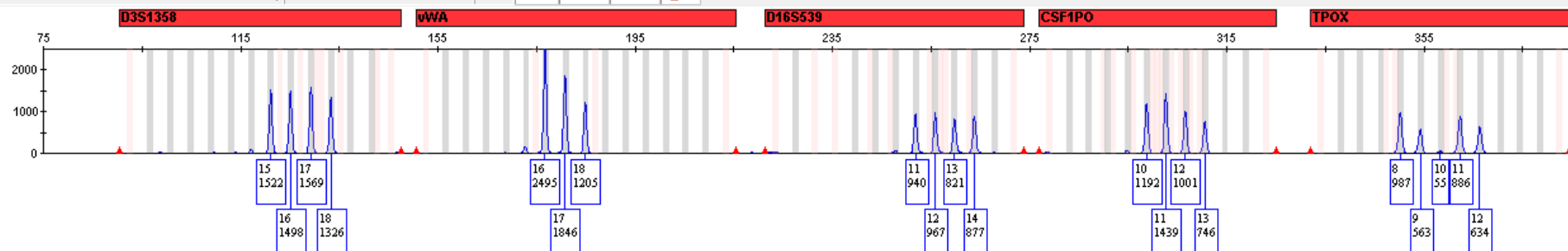
DE 1.1 SF_D01_GF_15s_2018-05-01-12-29-14_12 DE 1.1 SF

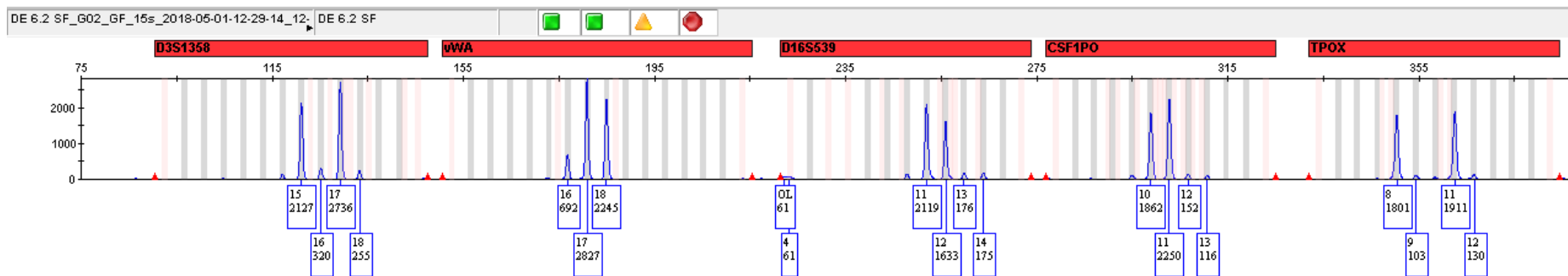
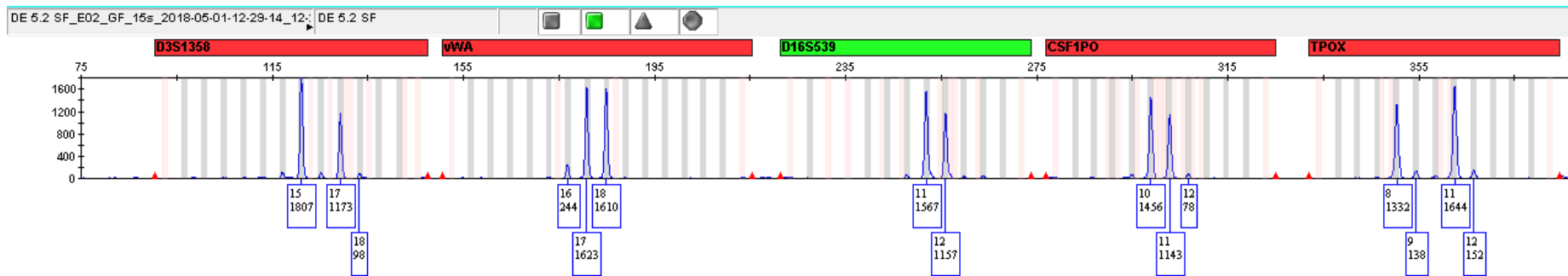
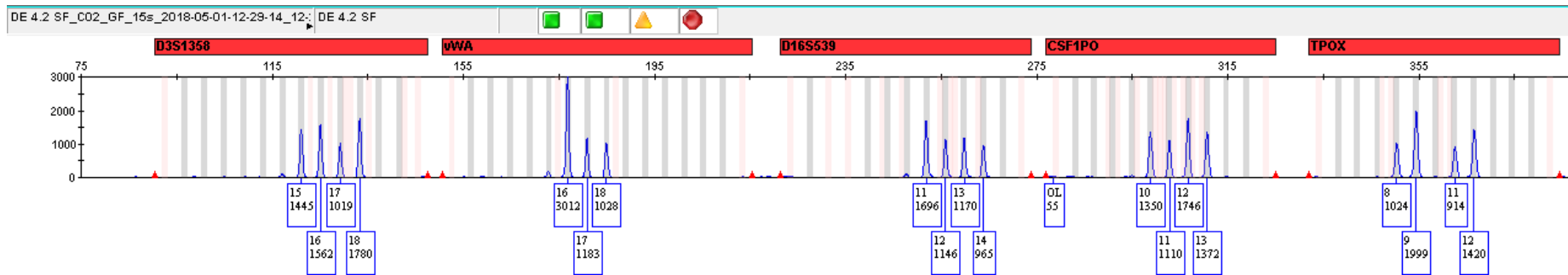


DE 2.1 SF_F01_GF_15s_2018-05-01-12-29-14_12 DE 2.1 SF



DE 3.1 SF_H01_GF_15s_2018-05-01-12-29-14_12 DE 3.1 SF





To Be Continued...

- Continued development of in-house differential extraction procedure for use with the AutoMate *Express*TM
- Further assessment of extreme variable elution volumes for research applications
- Full validation of purchased instrument
 - Sensitivity study, reproducibility and precision study, accuracy and concordance study, contamination assessment, mixture study, mock case samples

Acknowledgements

The Center for Forensic Science Research and Education

- Heather E. McKiernan
- Yih-Ling Saw



NMS Labs

- Dr. Heather Degnan
- Megan Foley



Arcadia University

- Dr. Shanan Tobe



Thermo Fisher Scientific

- Kim Wheeler
- Megan Meyer
- Bernie Scott





Thank you!

Questions?

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